Figure 1

```
                    10        20        30        40        50
                     |         |         |         |         |
XYN2_TRIRE   ---MVSFTSLLAASPP-SRASCRPAAEV---ESVAVEKRQTIQ------P
XYN1_HUMIN   ---MVSLKSVLAAATAVSSAIAAPFDFVPRDNSTALQARQVTP------N
XYNA_BACST   ----------------------MKLKKKMLTLLLTASMSFGLF------G
XYN1_TRIRE   ---MVAFSSLICALTSIASTLAMPTGLEPESSVNVTERGMYDFVLGAHND
XYN1_ASPAW   ---------------MKVTAAFAGLLVTAFAAPVPEPVLVS--------
XYN2_BACST   MCSSIPSLREVFANDFRIGAAVNPVTLEAQQSLLIRHVNSLTAENHMKFE

                    60        70        80        90        100
                     |         |         |         |         |
XYN2_TRIRE   GTGYNNGYFYSYWNDGHGGVTYTNGPGGQFSVNWS--NSG-NFVGGKGWQ
XYN1_HUMIN   AEGWHNGYFYSWWSDGGGQVQYTNLEGSRYQVRWR--NTG-NFVGGKGWN
XYNA_BACST   ATSSAATDYWQYWTDGGGMVNAVNGPGGNYSVTWQ--NTG-NFVVGKGWT
XYN1_TRIRE   HRRRASINYDQNYQTG-GQVSYSPSNTG-FSVNWN--TQD-DFVVGVGWT
XYN1_ASPAW   --RSAGINYVQNYNGNLGDFTYDESAGT-FSMYWEDGVSS-DFVVGLGWT
XYN2_BACST   HLQPEEGRFTFDIAIKSSTSPFSSHGVRGHTLVWHNQTPSWVFQDSQGHF

                    110       120       130       140       150
                     |         |         |         |         |
XYN2_TRIRE   PGTKNKVINFS-GSYNPNGNSYLSVYGWSRNPLIEYYIVENF---GTYNP
XYN1_HUMIN   PGT-GRTINYG-GYFNPQGNGYLAVYGWTRNPLVEYYVIESY---GTYNP
XYNA_BACST   VGSPNRVINYNAGIWEPSGNGYLTLYGWTRNALIEYYVVDSW---GTYRP
XYN1_TRIRE   TGS-SAPINFGGSFSVNSGTGLLSVYGWSTNPLVEYYIMED-----NHNY
XYN1_ASPAW   TGS-SNAITYSAEYSASGSSSYLAVYGWVNYPQAEYYIVEDY---GDYNP
XYN2_BACST   VGRDVLLERMKSHISTVVQRYKGKVYCWDVINEAVADEGSEWLRSSTWRQ

                    160       170       180       190       200
                     |         |         |         |         |
XYN2_TRIRE   STGATKLGEVTSDGSVYDIYRTQRVNQPSIIGTATFYQYWSVRRNHRSSG
XYN1_HUMIN   GSQAQYKGTFYTDGDQYDIFVSTRYNQPSIDGTRTFQQYWSIRKNKRVGG
XYNA_BACST   T--GNYKGTVNSDGGTYDIYTTMRYNAPSIDGTQTFQQFWSVRQSKRPTG
XYN1_TRIRE   PAQGTVKGTVTSDGATYTIWENTRVNEPSIQGTATFNQYISVRNSPRTSG
XYN1_ASPAW   CSSATSLGTVYSDGSTYQVCTDTRTNEPSITGTSTFTQYFSVRESTRTSG
XYN2_BACST   IIGDDFIQQAFLYAHEADPEALLFYNDYNECFPEKREKIYTLVKSLRDKG
```

Figure 1

```
                    210       220       230       240       250
                     |         |         |         |         |
XYN2_TRIRE   S----VNTANHFNAWA-QQGLTLGTMD-YQIVAVEGYFSSGSASITVS--
XYN1_HUMIN   S----VNMQNHFNAWQ-QHGMPLGQHY-YQVVATEGYQSSGESDIYVQTH
XYNA_BACST   SNV-SITFSNHVNAWR-SKGMNLGSSWAYQVLATEGYQSSGRSNVTVW--
XYN1_TRIRE   T----VTVQNHFNAWA-SLGLHLGQMN-YQVVAVEGWGGSGSASQSVSN-
XYN1_ASPAW   T----VTVANHFNFWA-QHGFGNSDFN-YQVMAVEAWSGAGSASVTISS-
XYN2_BACST   IPIHGIGMQAHWSLNRPTLDEIRAAIERYASLGVILHITELDISMFEFDD

                    260       270       280       290       300
                     |         |         |         |         |
XYN2_TRIRE   ----------------------------------------------------
```

Figure 1

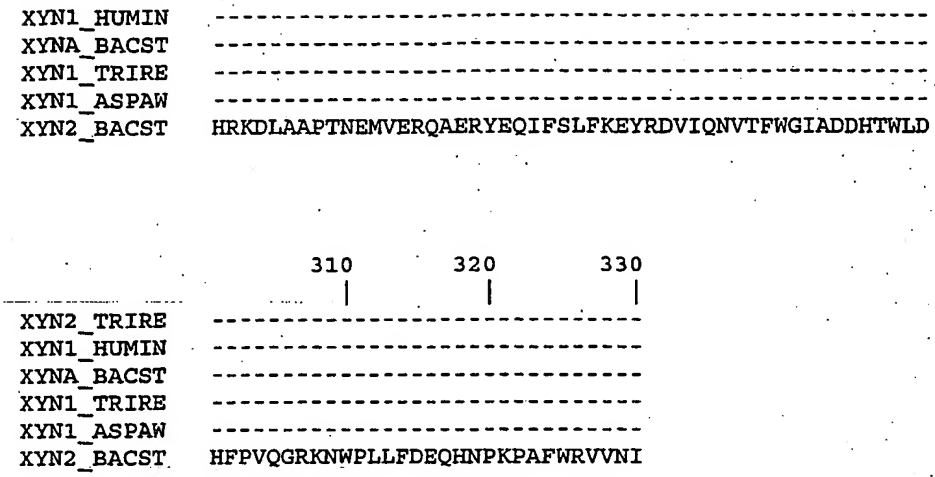
XYN1_HUMIN
XYNA_BACST
XYN1_TRIRE
XYN1_ASPAW
XYN2_BACST HRKDLAAPTNEMVERQAERYEQIFSLFKEYRDVIQNVTFWGIADDHTWLD
310
320
330
XYN2_TRIRE
XYN1_HUMIN
XYNA_BACST
XYN1_TRIRE
XYN1_ASPAW
XYN2_BACST HFPVQGRKNWPLLFDEQHNPKPAFWRVVNI

FIGURE 2

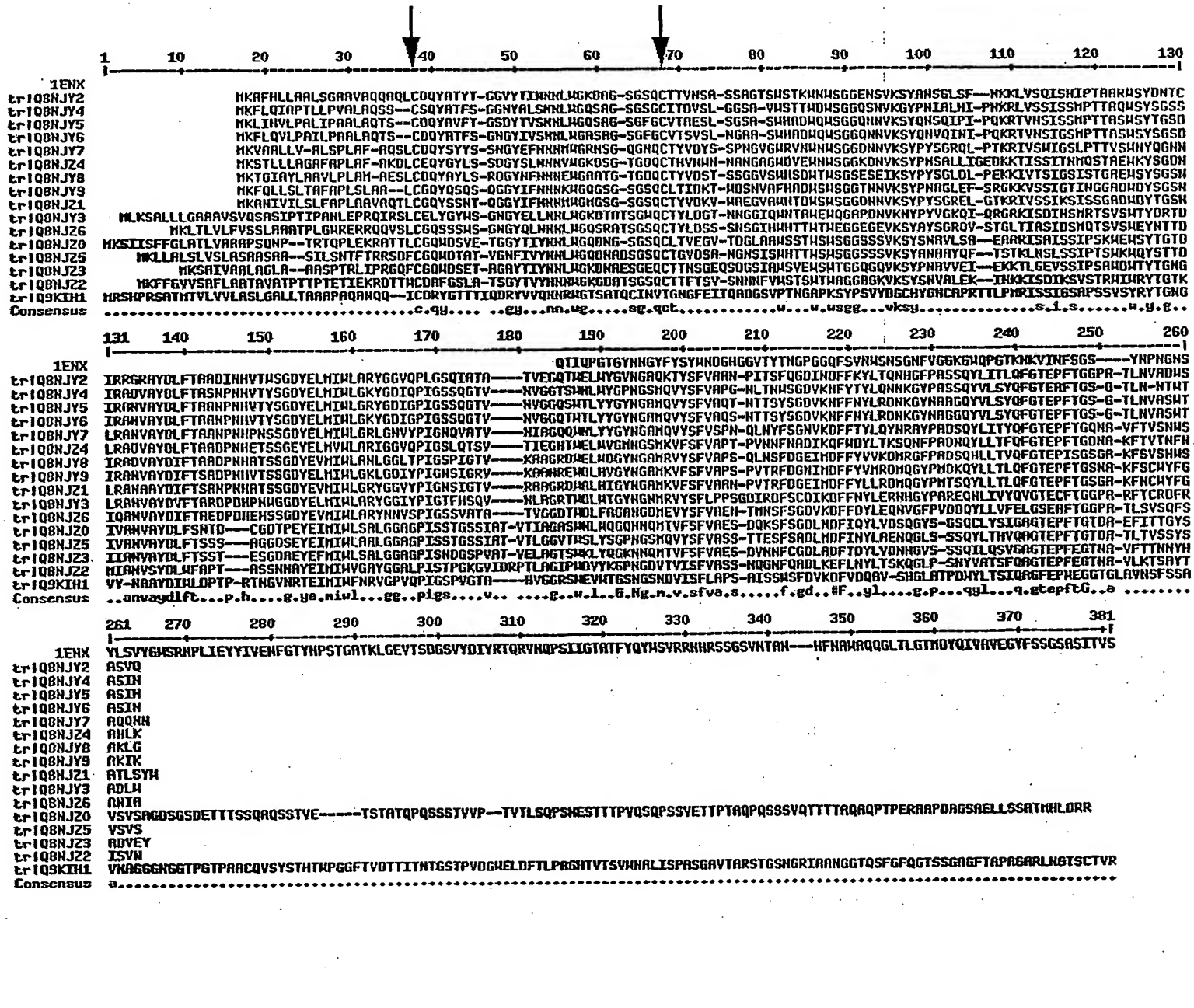

Figure 3

H22K	5′- GAACGATGGCAAGGGCGGCGTGACG -3′
S65C	5′- CTTCTCGGGCTGCTACAACCCAAACGG -3′
N92C	5′- ACATCGTCGAGTGTTTTGGCACCTAC -3′
F93W	5′- CATCGTCGAGAACTGGGGCACCTACAACC -3′
N97R	5′- GGCACCTACCGACCGTCCACG -3′
V108H	5′- CAAGCTGGGCGAGCACACCTCCGAC -3′
H144C	5′- CGCCGCAACTGTCGCTCGAGC -3′
F180Q	5′- GTGGAGGGTTACCAAAGCTCTGGCTCTGC -3′
S186C	5′- TCTGGCTCTGCTTGCATCACCGTCAGC -3′
T2C	5′-GAGAAGCGCCAGTGCATTCAGCCCGGC-3′
T28C	5′-GTGACGTACTGCAATGGTCCCGGCGGG-3′
K58R	5′-GGCACCAAGAACAGGGTCATCAACTTCTCGGGC-3′
191D	5′-TCCATCACCGTCAGCGATTAAAGGGGGCTCTTC-3′
P5C	5′-CCCAGACGATTCAGTGCGGCACGGGCTACAAC-3′
N19C	5′-CTTCTACTCGTACTGGTGCGATGGCCACGGCG-3′
T7C	5′-CGATTCAGCCCGGCTGCGGCTACAACAACGGC-3′
S16C	5′-CAACGGCTACTTCTACTGCTACTGGAACGATGGCC-3'
N10C	5′-CCGGCACGGGCTACTGCAACGGCTACTTCTACTC-3'
N29C	5′-GGCGTGACGTACACCTGCGGTCCCGGCGGGC-3'
L105C	5′-GGCGCCACCAAGTGCGGCGAGGTCACC-3'
Q162C	5′-GCGTGGGCTCAGTGCGGCCTGACGCTCG-3'

Figure 4.

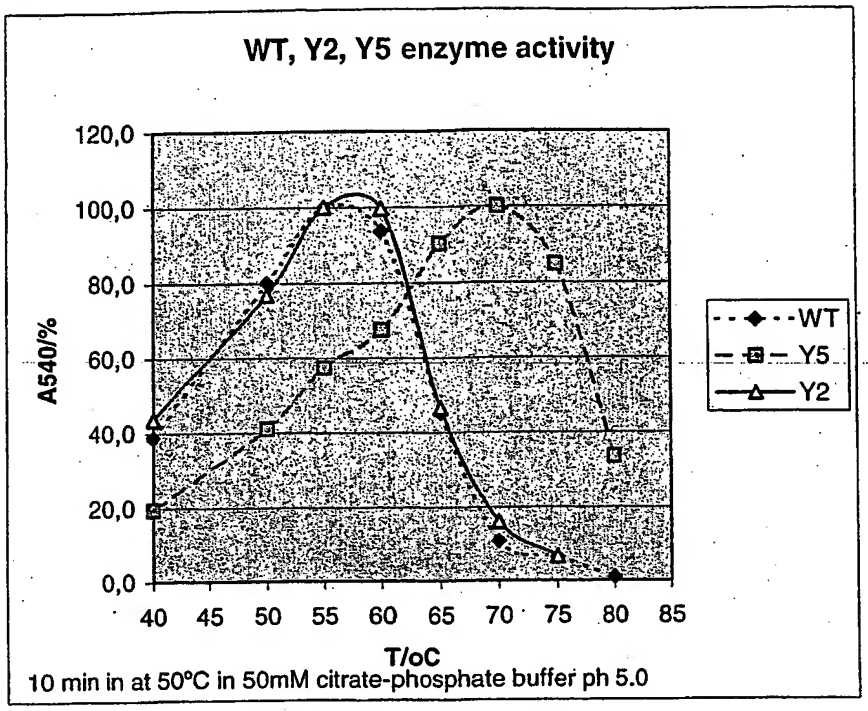
WT, Y2, Y5 enzyme activity
120,0
100,0
80,0
60,0
40,0
20,0
0,0
A540/%
40 45 50 55 60 65 70 75 80 85
T/oC
WT
Y5
Y2
10 min in at 50°C in 50mM citrate-phosphate buffer ph 5.0

Figure 5

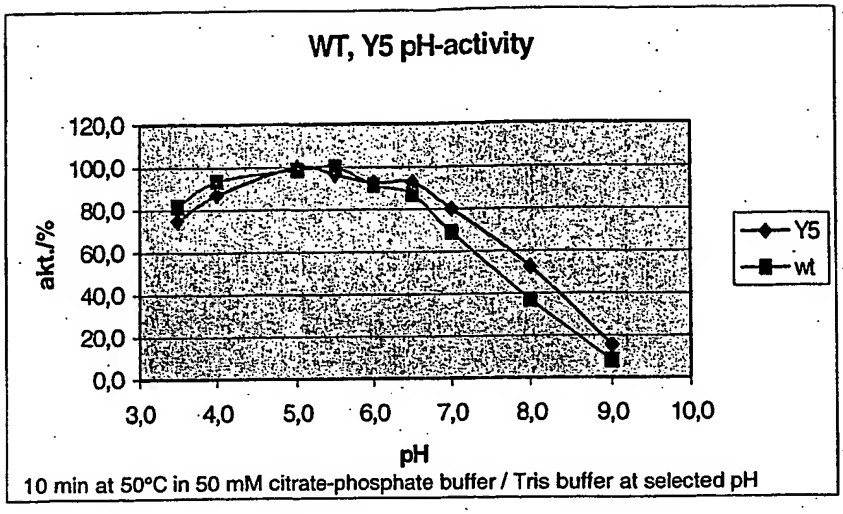
WT, Y5 pH-activity
120,0
100,0
80,0
60,0
40,0
20,0
0,0
akt./%
3,0
4,0
5,0
6,0
7,0
8,0
9,0
10,0
pH
Y5
wt
10 min at 50°C in 50 mM citrate-phosphate buffer / Tris buffer at selected pH

Figure 6

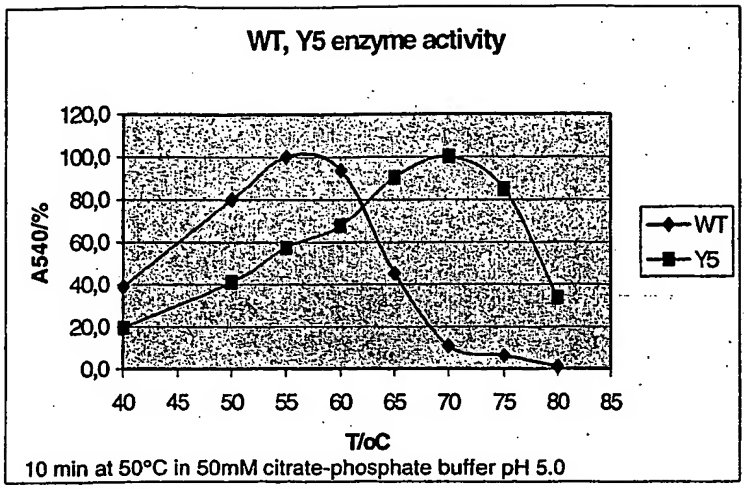
WT, Y5 enzyme activity
120,0
100,0
80,0
60,0
40,0
20,0
0,0
A540/%
40 45 50 55 60 65 70 75 80 85
T/oC
WT
Y5
10 min at 50°C in 50mM citrate-phosphate buffer pH 5.0

Figure 7

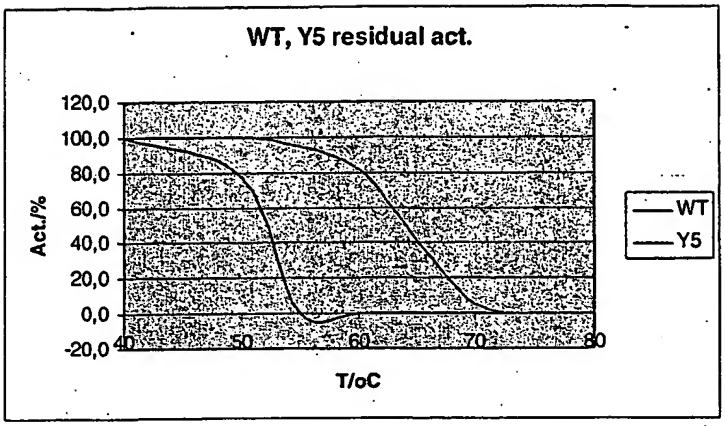
WT, Y5 residual act.
120,0
100,0
80,0
60,0
40,0
20,0
0,0
-20,0
Act./%
40
50
60
70
80
T/oC
WT
Y5

Figure 8

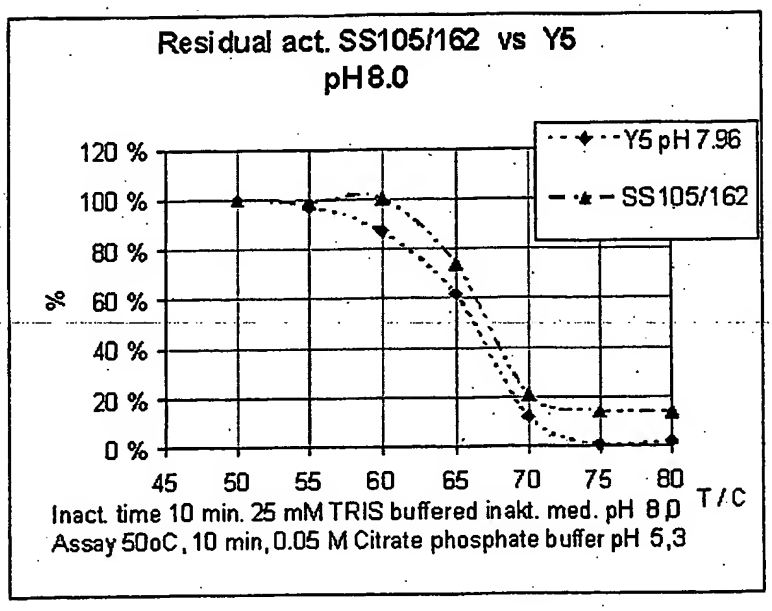
Residual act. SS105/162 vs Y5
pH8.0
Y5 pH 7.96
SS105/162
120 %
100 %
80 %
60 %
40 %
20 %
0 %
%
45
50
55
60
65
70
75
80
T/C
Inact. time 10 min. 25 mM TRIS buffered inakt. med. pH 8,0
Assay 50oC, 10 min, 0.05 M Citrate phosphate buffer pH 5,3

Figure 9.

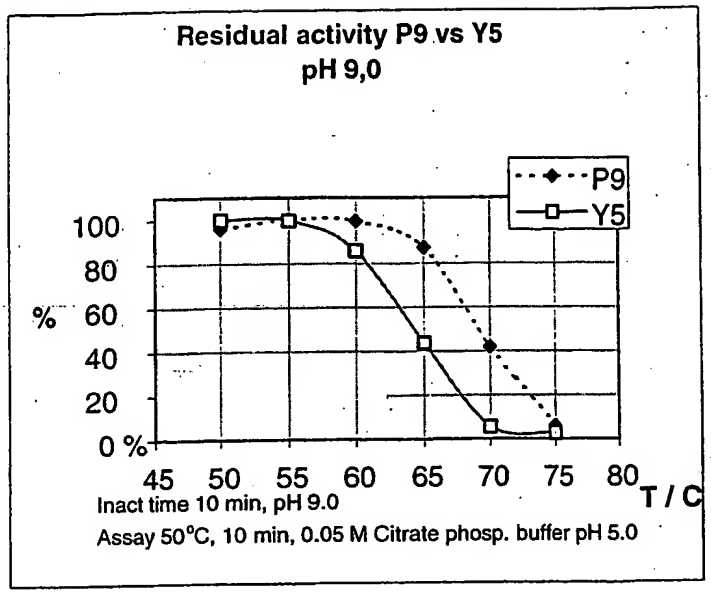
Residual activity P9 vs Y5
pH 9,0
P9
Y5
100
80
%
60
40
20
0 %
45
50
55
60
65
70
75
80
T / C
Inact time 10 min, pH 9.0
Assay 50°C, 10 min, 0.05 M Citrate phosp. buffer pH 5.0

Figure 10.

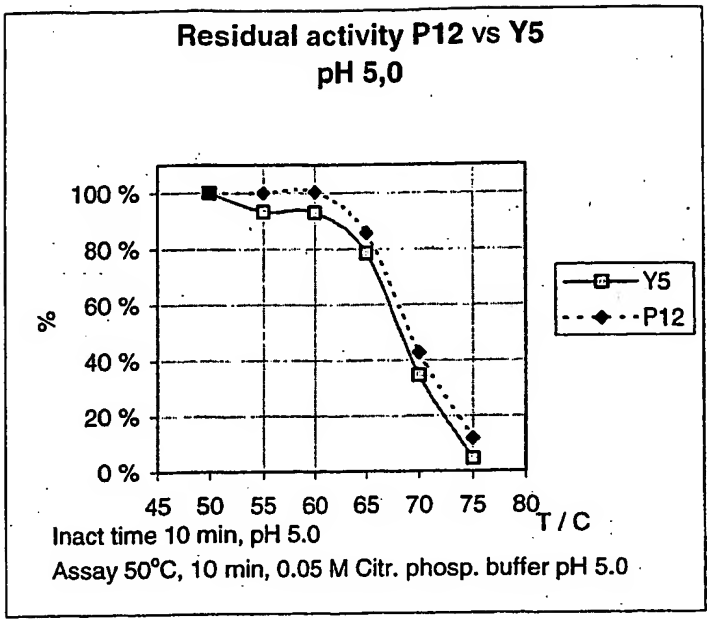
Residual activity P12 vs Y5
pH 5,0
100 %
80 %
60 %
40 %
20 %
0 %
%
45 50 55 60 65 70 75 80
T / C
Y5
P12
Inact time 10 min, pH 5.0
Assay 50°C, 10 min, 0.05 M Citr. phosp. buffer pH 5.0

Figure 11.

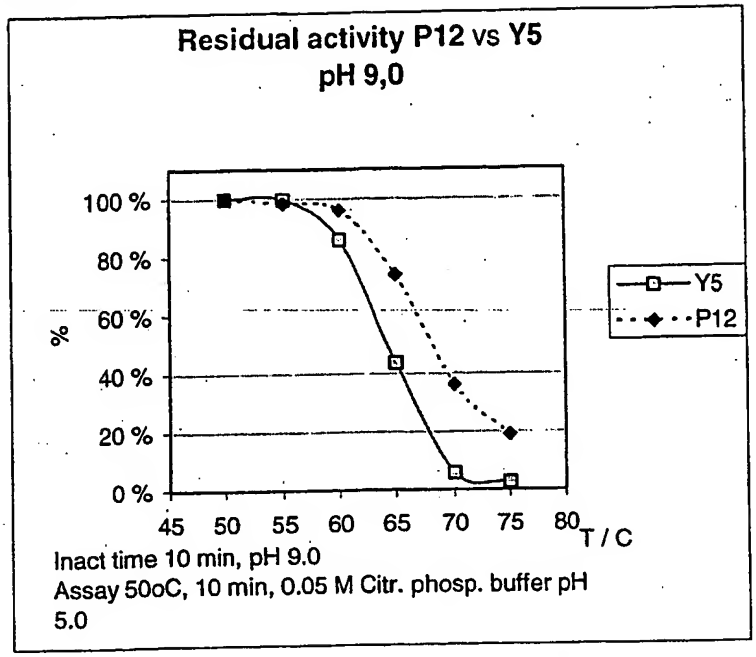
Residual activity P12 vs Y5
pH 9,0
100 %
80 %
60 %
40 %
20 %
0 %
%
45 50 55 60 65 70 75 80
T / C
Y5
P12
Inact time 10 min, pH 9.0
Assay 50oC, 10 min, 0.05 M Citr. phosp. buffer pH 5.0

Figure 12.

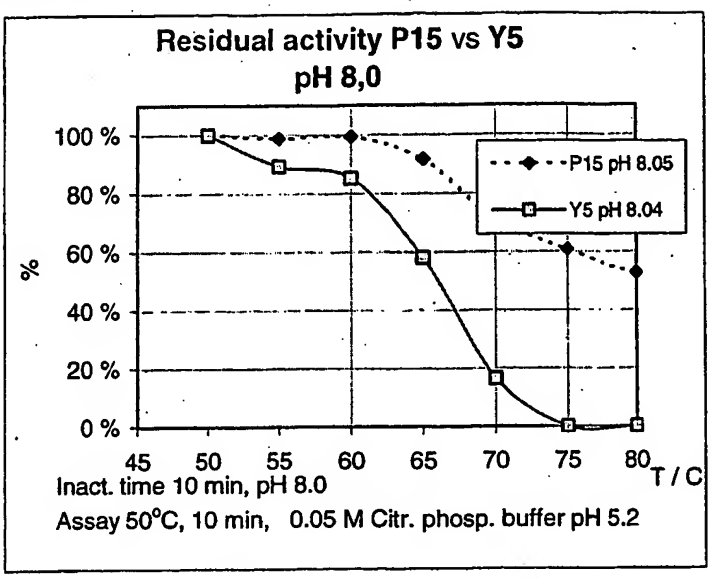
Residual activity P15 vs Y5
pH 8,0
100 %
80 %
60 %
40 %
20 %
0 %
%
45
50
55
60
65
70
75
80
T / C
P15 pH 8.05
Y5 pH 8.04
Inact. time 10 min, pH 8.0
Assay 50°C, 10 min, 0.05 M Citr. phosp. buffer pH 5.2

Figure 13.

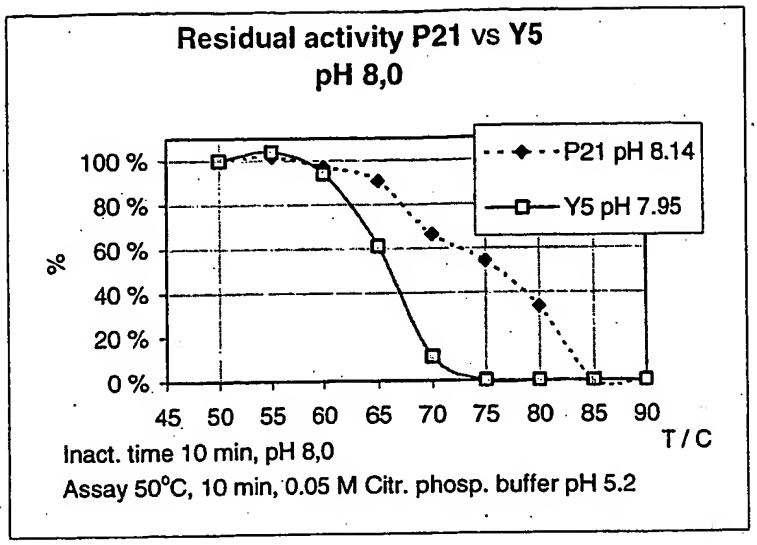
Residual activity P21 vs Y5
pH 8,0
P21 pH 8.14
Y5 pH 7.95
100 %
80 %
60 %
40 %
20 %
0 %
%
45 50 55 60 65 70 75 80 85 90
T / C
Inact. time 10 min, pH 8,0
Assay 50°C, 10 min, 0.05 M Citr. phosp. buffer pH 5.2

Figure 14.

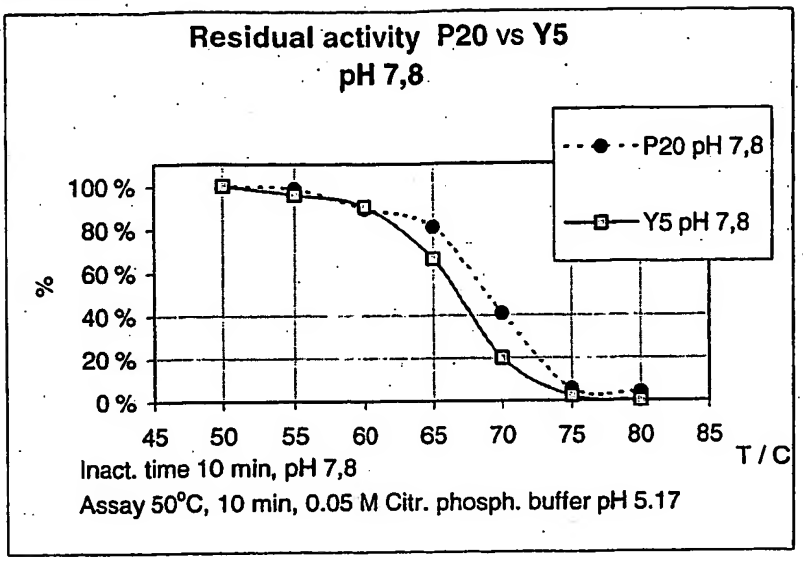
Residual activity P20 vs Y5
pH 7,8
P20 pH 7,8
Y5 pH 7,8
100 %
80 %
60 %
40 %
20 %
0 %
%
45 50 55 60 65 70 75 80 85
T / C
Inact. time 10 min, pH 7,8
Assay 50°C, 10 min, 0.05 M Citr. phosph. buffer pH 5.17

Figure 15.

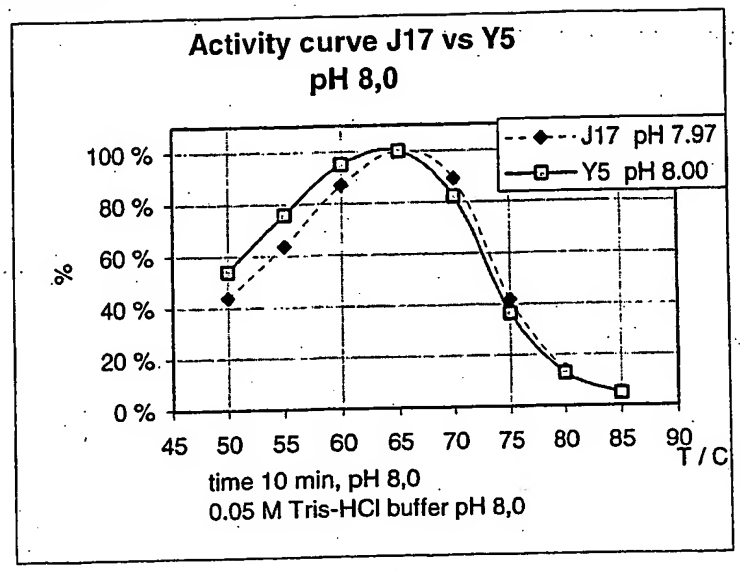
Activity curve J17 vs Y5
pH 8,0
J17 pH 7.97
Y5 pH 8.00
100 %
80 %
60 %
40 %
20 %
0 %
%
45 50 55 60 65 70 75 80 85 90
T / C
time 10 min, pH 8,0
0.05 M Tris-HCl buffer pH 8,0

Figure 16.

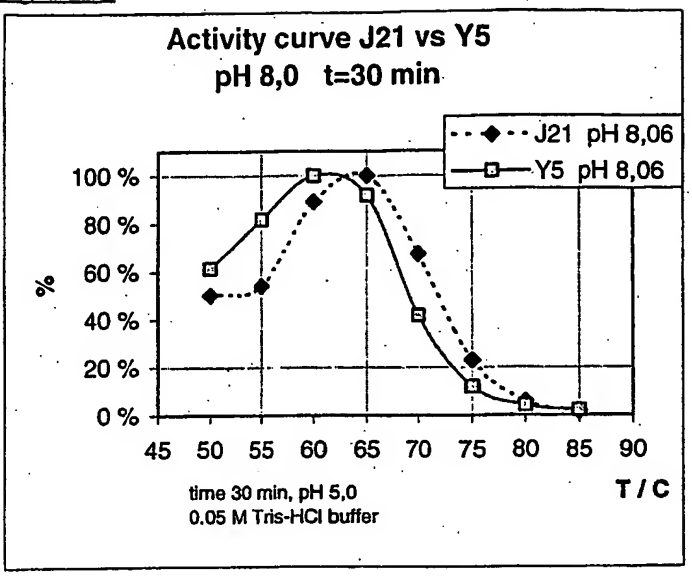
Activity curve J21 vs Y5
pH 8,0 t=30 min
J21 pH 8,06
Y5 pH 8,06
100 %
80 %
60 %
40 %
20 %
0 %
%
45 50 55 60 65 70 75 80 85 90
T / C
time 30 min, pH 5,0
0.05 M Tris-HCl buffer

Figure 17

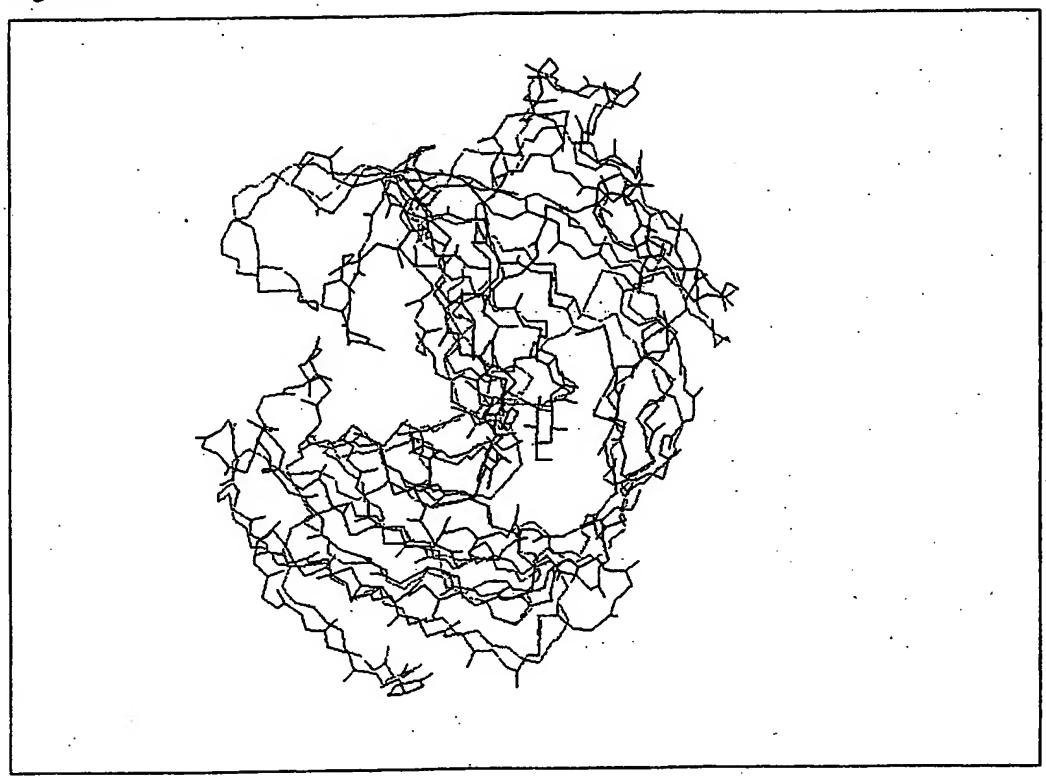

Figure 18

Trichoderma reesei Xyl II protein (high pI xylanase)
the full sequence, including signal and pro sequence

MVSFTSLLAGVAAISGVLAAPAAEVESVAVEKRQTIQPGTGYNNGYFYSYWNDGHGGVTYTNGPGGQFSVNWSNSGNFVG
GKGWQPGTKNKVINFSGSYNPNGNSYLSVYGWSRNPLIEYYIVENFGTYNPSTGATKLGEVTSDGSVYDIYRTQRVNQPS
IIGTATFYQYWSVRRNHRSSGSVNTANHFNAWAQQGLTLGTMDYQIVAVEGYFSSGSASITVS (SEQ. ID NO: 1)

Trichoderma reesei XynII gene (high pI xylanase)
DNA from start codon to stop codon (includes a single intron)

ATGGTCTCCTTCACCTCCCTCCTCGCCGGCGTCGCCGCCATCTCGGGCGTCTTGGCCGCTCCCGCCGCCGAGGTCGAATC
CGTGGCTGTGGAGAAGCGCCAGACGATTCAGCCCGGCACGGGCTACAACAACGGCTACTTCTACTCGTACTGGAACGATG
GCCACGGCGGCGTGACGTACACCAATGGTCCCGGCGGGCAGTTCTCCGTCAACTGGTCCAACTCGGGCAACTTTGTCGGC
GGCAAGGGATGGCAGCCCGGCACCAAGAACAAGTAAGACTACCTACTCTTACCCCCTTTGACCAACACAGCACAACACAA
TACAACACATGTGACTACCAATCATGGAATCGGATCTAACAGCTGTGTTTTCAAAAAAAAGGGTCATCAACTTCTCGGGC
AGCTACAACCCCAACGGCAACAGCTACCTCTCCGTGTACGGCTGGTCCCGCAACCCCCTGATCGAGTACTACATCGTCGA
GAACTTTGGCACCTACAACCCGTCCACGGGCGCCACCAAGCTGGGCGAGGTCACCTCCGACGGCAGCGTCTACGACATTT
ACCGCACGCAGCGCGTCAACCAGCCGTCCATCATCGGCACCGCCACCTTTTACCAGTACTGGTCCGTCCGCCGCAACCAC
CGCTCGAGCGGCTCCGTCAACACGGCGAACCACTTCAACGCGTGGGCTCAGCAAGGCCTGACGCTCGGGACGATGGATTA
CCAGATTGTTGCCGTGGAGGGTTACTTTAGCTCTGGCTCTGCTTCCATCACCGTCAGCTAA (SEQ. ID NO: 2)

Figure 19

Trichoderma reesei EGL III protein (endoglucanase III)
the full sequence, including signal sequence

MKFLQVLPALIPAALAQTSCDQWATFTGNGYTVSNNLWGASAGSGFGCVTAVSLSGGASWHADWQWSGGQNNVKSYQNSQ
IAIPQKRTVNSISSMPTTASWSYSGSNIRANVAYDLFTAANPNHVTYSGDYELMIWLGKYGDIGPIGSSQGTVNVGGQSW
TLYYGYNGAMQVYSFVAQTNTTNYSGDVKNFFNYLRDNKGYNAAGQYVLSYQFGTEPFTGSGTLNVASWTASIN
(SEQ. ID NO: 3)

Trichoderma reesei EG III gene (endoglucanase III)
DNA from start codon to stop codon (includes two introns)

ATGAAGTTCCTTCAAGTCCTCCCTGCCCTCATACCGGCCGCCCTGGCCCAAACCAGCTGTGACCAGTGGGCAACCTTCAC
TGGCAACGGCTACACAGTCAGCAACAACCTTTGGGGAGCATCAGCCGGCTCTGGATTTGGCTGCGTGACGGCGGTATCGC
TCAGCGGCGGGGCCTCCTGGCACGCAGACTGGCAGTGGTCCGGCGGCCAGAACAACGTCAAGTCGTACCAGAACTCTCAG
ATTGCCATTCCCCAGAAGAGGACCGTCAACAGCATCAGCAGCATGCCCACCACTGCCAGCTGGAGCTACAGCGGGAGCAA
CATCCGCGCTAATGTTGCGTATGACTTGTTCACCGCAGCCAACCCGAATCATGTCACGTACTCGGGAGACTACGAACTCA
TGATCTGGTAAGCCATAAGAAGTGACCCTCCTTGATAGTTTCGACTAACAACATGTCTTGAGGCTTGGCAAATACGGCGA
TATTGGGCCGATTGGGTCCTCACAGGGAACAGTCAACGTCGGTGGCCAGAGCTGGACGCTCTACTATGGCTACAACGGAG
CCATGCAAGTCTATTCCTTTGTGGCCCAGACCAACACTACCAACTACAGCGGAGATGTCAAGAACTTCTTCAATTATCTC
CGAGACAATAAAGGATACAACGCTGCAGGCCAATATGTTCTTAGTAAGTCACCCTCACTGTGACTGGGCTGAGTTTGTTG
CAACGTTTGCTAACAAAACCTTCGTATAGGCTACCAATTTGGTACCGAGCCCTTCACGGGCAGTGGAACTCTGAACGTCG
CATCCTGGACCGCATCTATCAACTAA (SEQ. ID NO: 4)

Figure 20

atggttgccttttccagcctcatctgcgctctcaccagcatcgccagtactctggcgatgcccacaggcctcgagcctgagagcagtgtcaacgtcacagagcgtggcatgtacgactttgttctt
ggagctcacaatgatcatcgccgtcgtgctagcatcaactacgaccaaaactaccaaactggcggacaagtcagctattcgccttccaacactggcttctcagtgaactggaacactcaagatg
actttgttgtgggcgttggttggacgactggatcttctgcgtaggaggactcctcatcattctgcactttgaaagcatcttctgaccaaaagcttctcttagtcccatcaactttggcggctcttttagtg
tcaacagcggaactggcctgctttccgtctatggctggagcaccaacccactggttgagtactacatcatggaggacaaccacaactacccagcacagggtaccgtcaagggaaccgtcacc
agcgacggagccacttacaccatctgggagaatacccgtgtcaacgagccttccatccagggcacagcgaccttcaaccagtacatttccgtgcggaactcgcccaggaccagcggaactg
ttactgtgcagaaccacttcaatgcttgggcctcgcttggcctgcaccttgggcagatgaactaccaggttgtcgctgtcgaaggctggggtggtagtggttctgcctcacagagtgtcagcaac
tag

Xylanase I Amino Acid sequence

MVAFSSLICALTSIASTLAMPTGLEPESSVNVTERGMYDFVLGAHNDHRRRASINYDQNYQTGGQVSYSPSNTGFSVNW
N
TQDDFVVGVGWTTGSSA.EDSSSFCTLKASSDQKLLLVPSTLAALLVSTAELACFPSMAGAPTHWLSTTSWRTTTTTQHR
VPSREPSPATEPLTPSGRIPVSTSLPSRAQRPSTSTFPCGTRPGPAELLLCRTTSMLGPRLACTLGR.TTRLSLSKAGVV
VVLPHRVSAT